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Original Article

Reproducibility of nasal function measurements with histamine and adenosine monophosphate nasal challenge testing in patients with allergic rhinitis

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Abstract

Background: Adenosine monophosphate (AMP) acts by releasing inflammatory mediators from mast cells and may be used for bronchial and nasal provocation tests. The aim of the present study was to determine whether AMP could be used in a dose–response manner to evaluate nasal function and to evaluate the reproducibility of nasal function measurements with nasal challenge testing using histamine and AMP in patients with perennial allergic rhinitis.

Methods: Nine patients were challenged on three separate occasions for each challenge with doubling doses of either histamine (0.25–8 mg/mL) or AMP (25–800 mg/mL). Challenge measurements were made of peak inspiratory flow rate (PIFR), acoustic rhinometry (AR) and rhinomanometry (Rhino). The provocation concentration (PC_{30}) was calculated in order to produce: (i) a 30% fall in PIFR; (ii) a 30% fall in AR and (iii) a 30% increase in nasal airway resistance in Rhino and a symptom score of 10 (of 40). The mean intrasubject coefficient of variation (CV) was calculated for baseline and the corresponding PC.

Results: Baseline CV prior to histamine were 15.1, 19.6 and 15.8 for PIFR, AR and Rhino, respectively; prior to AMP, baseline CV were 12.7, 19.6 and 10.6% for PIFR, AR and Rhino, respectively. For histamine challenge, the PC_{30} were 26.5, 27.4 and 38.7% for PIFR, AR and Rhino, respectively. For AMP challenge, the PC_{30} CV values were 46.2, 30.8 and 49.5% for PIFR, AR and Rhino, respectively. There was no significant difference in the provocative dose required to cause a predetermined change in response or the response at 1 mg/mL histamine and 100 mg/mL AMP.

Conclusions: Adenosine monophosphate may be used as a challenge agent for nasal challenge testing, although it results in greater variability than histamine.

Key words: adenosine monophosphate, allergic rhinitis, histamine, nasal challenge, nasal inspiratory flow.

Introduction

One component of mucosal inflammation is that of hyperresponsiveness, a reaction to contact of substances at lower concentrations than would normally be the case. The substance used for the nasal provocation tests can be considered as 'specific', such as allergens[1] or aspirin,[2,3] or 'non-specific', such as histamine.[4] Histamine is an example of a direct challenge test because it acts directly on histamine receptors in the nasal mucosa. It is less representative of the clinical situation than indirect challenges with, for example, allergen. Allergen challenges are popular in diagnosing specific allergy and have been shown to be more sensitive to skin prick testing.[5,6] However, these challenges assess a specific allergen and cannot be used for assessing the effect of therapy on a mixed population with allergies to different allergens. Recently, Polosa *et al*.[7] have published data showing that challenging the nasal mucosa with adenosine monophosphate (AMP), an indirect stimulus, results in histamine release from primed mast cells in atopic

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subjects. Adenosine monophosphate bronchial challenge has been confirmed to be a useful method of assessing asthma[8] and it is possible that AMP nasal challenge may have a role in assessing upper airway disease. However, prior to proceeding with studies assessing the ability of AMP nasal challenge in determining nasal inflammation and response to treatment, it is necessary to determine whether a dose–response effect with AMP exists and which doses should be used.

With nasal challenge testing, responses to stimuli are normally assessed by measuring nasal blockage, nasal discharge and sneezing.[9] Although the response can be calculated by weighing handkerchiefs and counting the number of sneezes, it is the obstructive component that is most easily and reproducibly measured.[1,10] This can be measured by rhinomanometry, acoustic rhinometry (AR) and nasal flow, although there is debate as to the optimal measure. Rhinomanometry is commonly used,[11–13] although some authors prefer AR.[14] Acoustic rhinometry has been compared with rhinomanometry by body plethsymography,[15] anterior rhinometry[16] and posterior rhinomanometry[17] during nasal challenge tests. In all these studies, rhinomanometry was as sensitive as AR; however, Roithmann *et al*.[15] and Austin and Foreman[17] have suggested that AR is easier to perform. Peak inspiratory flow rate (PIFR) can also be used to assess the response to stimulus in terms of challenge testing[10] and has been shown to be as sensitive as AR.[18] In the present study, we wished to evaluate the coefficient of variation (CV) by using PIFR, AR, rhinomanometry and symptoms at the same time. We chose to use posterior rhinomanometry because it assesses the total nasal resistance

METHODS

Patients

Nine patients (four females) with perennial allergic rhinitis, according to current criteria,[19] mean (± SEM) age 34.4 ± 4.2 years, were recruited into the study and underwent a histamine nasal challenge test and AMP challenge test. All patients had normal spirometry (mean forced expiratory volume in 1 s (FEV_1) 101 ± 2.8% predicted), seven patients were skin prick positive to grass, five were skin prick positive to house dust mite, five were skin prick positive to cat and four were skin prick positive to dog. Three patients were unable to perform the rhinomanometry due to technical reasons. There was a subgroup of six patients (two female), mean (± SEM) age 31.0 ± 3.6 years, FEV_1 105 ± 2.4% predicted, allergic to grass ($n = 4$), house dust mite ($n = 3$), cat ($n = 4$) and dog ($n = 3$) who performed all measurements; one additional person did not have evaluable results for AMP. Patients who were skin prick positive to a seasonal allergen performed the study out with the season for their allergen. One patient was taking oral loratadine prior to enrolment into the study, but no patient was taking intranasal corticosteroids. No subject had received oral corticosteroids or antibiotics for 6 months prior to the study. All subjects were non-smokers and had normal full blood count, biochemical profile and urinalysis. Approval for the study was obtained from the Tayside Medical Ethics Committee and all patients gave their written informed consent to participate.

Methodology

Patients attended the laboratory for each challenge on three separate occasions. Each occasion was separated by more than 3 days. No patient had received any medication for their perennial allergic rhinitis for at least 1 week prior to each study visit. All measurements at the study visit were conducted at the same time of day for each patient.

Measurements

Adenosine monophosphate nasal challenge

Patients had baseline measurements of nasal PIFR, AR, symptoms scoring and rhinomanometry. The above measurements were repeated 2 min after receiving a placebo nasal spray. Adenosine monophosphate was then administered via a nasal spray in doubling concentrations from 25 to 400 mg/mL, with the measurements repeated 2 min after each dose. The study was terminated on the request of the patient if the symptoms were severe or by the physician if the patient had an unrecordable PIFR or AR value. Patients were then offered topical xylometazoline (Otrivine; Norvartis Consumer Health, West Sussex, UK) and observed until their symptoms of nasal blockage subsided.

Nasal histamine challenge

Nasal histamine challenge was performed in the same manner as the AMP challenge. After a placebo baseline, patients were given doubling concentrations of histamine

from 0.25 to 8 mg/mL. The following measurements were performed 2 min after each concentration.

Nasal PIFR Nasal inspiratory flow rate was measured using an In-check™ flow meter (Clement Clarke International, Harlow, UK). After blowing their nose, patients inspired forcefully from residual volume to total lung capacity with their mouth closed. All measurements were made while in the sitting position with a good seal around a purpose-built facemask. The mean of three consecutive readings was recorded.

Rhinomanometry Patients had measurements of nasal resistance by posterior rhinomanometry using an NR6 rhinomanometer (GM Instruments, Ashgrove, Kilwinning, UK) with on-line computerized integration of total nasal flow and pressure change in a subgroup of six patients with histamine and five patients with AMP nasal challenge. Total nasal flow was measured with patients breathing tidal volumes through a facemask with their mouths closed. Nasal pressure was measured by placing a pressure probe in the patients' mouth with their soft palate open to represent posterior nasal pressure changes. Flow rates were calculated at a nasal pressure of 150 Pa.[20] The pressure transducer and flow meter were calibrated weekly.

Acoustic rhinometry Acoustic rhinometry was measured using a AI Executive acoustic rhinometer (GM Instruments). A probe was inserted 0.5 cm into each nostril such that a seal was obtained without distorting the nasal architecture. Patients were asked to hold their breath during the procedure and a probe stand was used in order to ensure correct positioning of the probe.[21] Measurements were of the minimum cross-sectional area (MCA) at the nasal valve (approximately 2 cm from nasal orifice). The total minimum area was taken to be sum of the measurement from the right and left nostrils.

Symptoms

Patients were asked to score their symptoms on an 11-point scale (0, no symptoms; 10, severe symptoms) in terms of 'runny nose', 'blocked/stuffy nose', 'itchy nose' and 'overall discomfort/feeling'. The total symptom score (out of 40) was the sum of the individual components.

Statistical analysis

Log dose–response curves were produced for each measurement. Provocation concentrations producing a 30% (PC_{30}) fall for nasal inspiratory flow rate and MCA and a 30% increase in nasal airways resistance were determined by interpolation of the curve. For total symptom score, the concentration required to cause a score of 10 was calculated from the log dose–symptom curve.

The intrasubject CV for repeated measures was calculated for AMP and histamine for each end-point. For AMP nasal challenge testing, the CV for rhinomanometry could only be calculated in three patients. Coefficients of variation were also calculated for baseline values and for the response after 1 mg/mL histamine and 100 mg/mL AMP.

Student's *t*-test was used to determine any significant difference between the baseline values and the value at 1 mg/mL histamine or 100 mg/mL AMP. Overall comparisons of the actual value of PC_{30} and CV for outcome measure after both AMP and histamine challenge were made by multifactorial analysis of variance using subject and outcome measure (PIFR, symptoms, AR) as factors. This was followed by Bonferroni's multiple range testing (set at 95% confidence interval (CI)) in order to obviate multiple pair-wise comparisons. Consequently, comparisons are only denoted as being significant ($P < 0.05$, two-tailed) or not significant. This analysis could not be performed for AMP challenge in the subgroup who performed rhinomanometry due to the small number of patients in this subgroup. The analysis was performed using Statgraphics statistical software package (STSC Software Publishing Group, Rockville, MD, USA) and Microsoft Excel 97 (Microsoft, Seattle, WA, USA).

RESULTS

It was possible to generate dose–response curves for both AMP and histamine using PIFR, AR, rhinomanometry and symptom scoring. The average dose–response curves for PIFR and AR are illustrated in Fig. 1 with a value of zero being assigned where recordings of PIFR and AR were below the detectable limit of the test.

It was not possible to generate a CV for provocation with AMP in three patients with AR, two patients with rhinomanometry and PIFR and symptoms and in one patient for symptoms with histamine. Overall comparisons of the actual value of PC_{30} and CV for outcome measure after both AMP and histamine challenge were not performed for rhinomanometry due to the small number of patients.

The response at baseline (i.e. prior to challenge) and at 1 mg/mL histamine and 100 mg/mL AMP are given in Table 1. There was no significant difference in baseline values or values after 1 mg/mL histamine and 100 mg/mL AMP for any measure. The provocation concentrations with both challenges for each outcome measurement are also shown in Table 1. For histamine challenge testing, there was a 0.1 (95% CI −1.6, 1.8) doubling dose difference for PIFR compared with symptoms and a 0.6 (95% CI −1.1, 2.3) dose difference for PIFR compared with AR. For AMP challenge, there was a 0.9 (95% CI −0.7, 2.5) doubling dose difference between PIF and symptoms and a 0.4 (95% CI −1.4, 2.1) doubling dose difference between PIFR and AR. There was no significant difference between the provocation concentration with AMP or histamine for any measure (Table 1).

The CV at baseline, after 1 mg/mL histamine and 100 mg/mL AMP and for the doubling dose difference is shown in Table 2. Although the CV for measurements tended to be greater with AMP challenge than with histamine challenge, especially for symptoms and PIFR, this was not statistically significant. For histamine challenge,

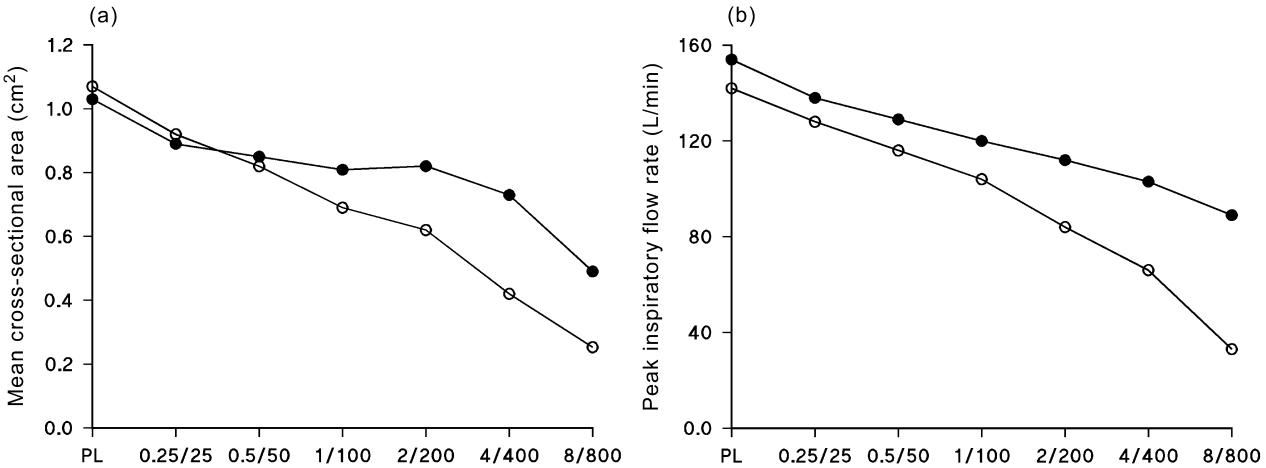

Fig. 1 (a) Average dose–response curves for minimal cross-sectional area as assessed by acoustic rhinometry for 0.25 and 25 mg/mL (0.25/25), 0.5 and 50 mg/mL (0.5/50), 1 and 100 mg/mL (1/100), 2 and 200 mg/mL (2/200), 4 and 400 mg/mL (4/400) and 8 and 800 mg/L (8/800) for histamine (●) and adenosine monophosphate (○), respectively. (b) Average dose–response curves for peak inspiratory flow rate.

Table 1 Baseline values prior to histamine and AMP nasal challenge, values after 1 mg/mL histamine and 100 mg/mL AMP and the provocative concentration of histamine and AMP causing a 30% fall in nasal peak inspiratory rate, a total symptom score of 10, a 30% fall in acoustic rhinometry minimal cross-sectional area and a 30% increase in airway resistance as measured by rhinomanometry

	PIFR	Symptom score	Acoustic rhinometry	Rhinomanometry
Histamine				
Baseline	147 ± 15 L/min	2.7 ± 1.5 units	1.1 ± 0.1 cm^2	198 ± 10 Pa/cm^3 per s
1 mg/mL	105 ± 13 L/min	10.6 ± 1.6 units	0.7 ± 0.1 cm^2	515 ± 103 Pa/cm^3 per s
Provocative concentration	1.03 ± 0.18 mg/mL	0.71 ± 0.55 mg/mL	0.67 ± 0.15 mg/mL	0.55 ± 0.16 mg/mL
AMP				
Baseline	153 ± 13 L/min	3.0 ± 1.4 units	1.0 ± 0.1 cm^2	180 ± 15 Pa/cm^3 per s
100 mg/mL	129 ± 16 L/min	9.9 ± 1.4 units	0.8 ± 0.1 cm^2	356 ± 69 Pa/cm^3 per s
Provocative concentration	180 ± 66 mg/mL	97 ± 22 mg/mL	125 ± 34 mg/mL	69 ± 22 mg/mL
P value*	0.11	0.80	0.13	0.69

Data show the mean ± SEM.

*The *P* values given are for the provocative concentration of AMP compared with histamine.

PIFR, peak inspiratory flow rate; AR, acoustic rhinometry minimal cross-sectional area.

Table 2 Mean intrasubject coefficient of variation for repeated measures at baseline prior to histamine and AMP nasal challenge and after 1 mg/mL histamine and 100 mg/mL AMP and for the provocative concentration of histamine and AMP causing a 30% fall in nasal peak inspiratory rate, a total symptom score of 10 and a 30% reduction in acoustic rhinometry minimal cross-sectional area and a 30% increase in airway resistance as measured by rhinomanometry

	PIFR	Symptom score	AR	Rhinomanometry
Histamine				
Baseline	15.1		19.6	15.8
1 mg/mL	23.1	22.9	24.8	32.6
Provocative concentration	26.5	24.4	27.4	38.7
AMP				
Baseline	12.7		19.6	10.6
100 mg/mL	21.7	37.8	20.3	39.0
Provocative concentration	46.2	41.9	30.8	49.5

PIFR, peak inspiratory flow rate; AR, acoustic rhinometry minimal cross-sectional area.

there was a 2.1% (95% CI –19, 23%) difference between PIFR and symptoms and a 0.9% (95% CI –19, 21%) difference between PIFR and AR. For AMP challenge, there was a 4.2% (95% CI –29, 36%) difference between PIFR and symptoms and a 15.3% (95% CI –25, 55%) difference between PIFR and AR.

Discussion

We have shown that it is possible to use AMP as the stimulus in a dose-ranging manner and to detect a dose–response effect by measuring PIFR, MCA using AR, airways resistance using rhinomanometry and symptom scoring. The provocation concentration for measurements with AMP was in the same order of magnitude to histamine when compared on a 100 : 1 basis and there was no difference in response after 100 mg/mL AMP and 1 mg/mL histamine. This suggests that the doses of AMP chosen were appropriate, although the present study was not designed to compare the response of AMP and histamine.

However, it was not possible to generate a PC_{30} or a CV for the nasal challenge in all patients with all end-points with both challenges. Furthermore, the CV for measurements with the AMP challenge tended to be greater than with the histamine challenge. However, if a non-specific indirect nasal challenge test is required for evaluation or research, then AMP nasal challenge may be suitable.

The CV was similar for all chosen end-points (i.e. AR, PIFR, rhinomanometry and symptoms). For this reason, it may be possible to use PIFR as a simple alternative to the more expensive laboratory measures of nasal function. This would mean that nasal challenge testing could be performed, on a screening basis, in the laboratory; however, larger studies would be required to validate this further. In this respect, we have shown recently that by using nasal PIFR, but not AR or rhinomanometry, as the end-point of nasal challenge testing with histamine, it was possible to detect a significant difference with treatment with intranasal mometasone.[22]

Pirila *et al*.[16] compared AR and rhinomanometry using allergen challenge and showed that the CV was lower with AR than rhinomanometry. However, in that study, anterior rather than posterior rhinomanometry was performed. Gleeson *et al*.[23] also compared PIFR, AR and rhinomanometry after low- (0.4%) and high-dose (0.8%) histamine and showed that there was comparable sensitivity and significant correlation between all methods.

With bronchial challenge testing, the standard cut-off used for a change in response is a 20% fall for FEV_1; however, there is no such standard value for the different measures of nasal function in nasal challenge testing. Pirila *et al*.[16] used a 15% decrease in MCA and a 50% increase in nasal resistance after 30 min, and 30 and 100% changes, respectively, after 60 min. Alternatively, with airway resistance, Gianico *et al*.[12] used 100% change and Kanthawatana *et al*.[13] suggested a 300% increase. When assessing measures of nasal flow, Plavec *et al*.[10] used a value of a 30% fall because this had been shown to be significant in a pilot study. We also used a 30% decrease in nasal peak inspiratory flow and showed that the concentration required was similar to that causing significant symptoms (total symptoms score of 10).

Our study was limited by the small sample size, although the study was designed as a proof of concept study and not to compare the effect of AMP and

histamine nasal challenge. The statistical analysis is for illustrative information only and the non-significant difference between AMP and histamine does not suggest that these challenges are equivalent. Furthermore, we did not assess healthy non-atopic volunteers, so that we cannot comment on sensitivity or specificity of the AMP nasal challenge test. However, because we have demonstrated that it is possible to use AMP as a stimulus for nasal challenge in a log dose–response manner, it is now possible to evaluate any theoretical benefits that nasal AMP challenge may have on assessing the degree of nasal inflammation and response to treatment with rhinitis therapy.

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